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(54) Title: HUMAN INFLUENZA VIRUS PEPTIDES BINDING HLA-T MOLECULE

(57) Abstract

A peptide comprising an amino acid sequence derived from a human influenza virus protein, such as the human influenza M protein, wherein said amino acid sequence has the ability to bind to a human Major Histocompatibility Complex Class I molecule. Its use in prophylactic or therapeutic treatment of a human influenza virus-related disease, or in a diagnostic test or assay.

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HUMAN INFLUENZA VIRUS PEPTIDES BINDING HLA-I MOLECULE

Field of the invention

The invention is concerned with novel peptides derived from a protein of the Human Influenza Virus, and their use in or for: 1) the induction of primary influenza-specific T cells that can be used for therapeutic treatment, in particular the induction of HLA class I-restricted $CD8^+$ cytotoxic T cells (CTL), and 2) pharmaceutical compositions for a prophylactic or therapeutic treatment of human individuals. All against diseases in which the influenza virus is playing a role.

Background of the invention

Influenza, an acute respiratory illness, is known to afflict humans since ancient times. The sudden appearance of epidemics of respiratory diseases persisting for a few weeks and equally sudden disappearance are characteristic. Epidemics vary in severity but usually cause mortality in the elderly. The virus has killed untold millions throughout the centuries and it has also altered the course of history. The severity of the disease accelerated the search for the causative agent. In 1933 the influenza virus was then first isolated from humans [ref 1]. The influenza virus belongs to the group of myxoviruses.

Influenza viruses are divided into types A, B and C based on the antigenic differences between their nucleoprotein (NP) and matrix (M; also called membrane) protein antigens. Influenza A viruses are further subdivided into subtypes and the revised nomenclature system includes the host of origin, geographical origin, strain number and year of isolation. Influenza A virus

is the most common variety of influenza causing viruses, and is responsible for the pandemics. Influenza B is usually associated with smaller and less virulent outbreaks. The C virus is found only rarely.

The structures of influenza viruses of types A, B, and C are similar. The hemagglutin (HA) and neuraminidase (NA) glycoproteins are attached by short sequences of hydrophobic amino acids to a lipid envelope derived from the plasma membrane of the host cell. Within the lipid envelope lies the M protein, which is believed to be structural in function. Within the matrix shell are eight single-stranded RNA molecules of negative sense associated with the NP and three large proteins (PB1, PB2 and PA) responsible for RNA replication and transcription. At least three virus-coded nonstructural proteins (NS1, NS2 and M2) are found in infected cells but their functions are unknown [ref 2]. The influenza virus-coded proteins and their major characteristics are shown in Table 1.

Table 1: Influenza virus-coded proteins

Designation	occurrence*	Characteristics
M1 or M	3000	Major virion component, surrounds the core, involved in assembly and budding
M2	20-60	Involved in assembly and budding
NP	1000	Internal protein associated with RNA & polymerase proteins, helical arrangement
PB1/PB2/PA	30-60	Internal proteins associated with RNA transcriptase activity
HA	500	Surface glycoprotein trimer responsible for attachment of virus to cells
NA	100	Surface glycoprotein, enzyme activity
NS1		Function unknown
NS2		Function unknown

* occurrence: approximate number of molecules per virus particle

It is variation in the HA glycoprotein which is mainly responsible for the recurring outbreaks of influenza and for our inability to control these by immunization [ref 3]. Cleavage of the HA polypeptide into HA1 and HA2 is necessary for the virus particles to be infectious.

The NP is one of the type-specific antigens of influenza viruses that distinguishes among the influenza type A, B, and C viruses. The influenza B and C NP proteins are larger (560-565 amino acids) than those of type A (498 amino acids). The NP is a multifunctional protein having a structural role in forming the NP complex and a role in transcription and replication. The NP protein may play a role in determining species specificity. Monoclonal antibodies to the NP do not provide passive protection and immunization with this protein induces very limited resistance to infection. However, the NP protein is a major antigen recognized by cytotoxic lymphocytes (CTL) and several of the epitopes recognized by the CTL are conserved in human influenza A viruses [ref 4, 5].

The M1 protein, which is 252 amino acids long, is a virion structural protein that is intimately associated with the lipid bilayer in such a manner that it is in close proximity to both glycoproteins and to the RNP complex [ref 2, 6]. In a typical influenza A virus, M1 is encoded in an essentially complete transcript of segment 7. It is believed to be a multifunctional protein also having a role in the downregulation of the activity of the virion-associated transcriptase and is located in the nucleus, cytoplasm and plasma membrane in infected cells [ref 7, 8]. Passively transferred monoclonal antibodies to this protein do not confer resistance to infection [ref 9]. Comparison of the sequences of RNA segment 7 of the H3N2 and H1N1 strains show that the sequences coding for the M1 protein of these viruses isolated 38 years apart are highly conserved in keeping with antigenic studies [ref 2]. Comparison of 230 nucleotides of RNA segment 7 from five human H1N1, H2N2 and H3N2 strains isolated over a 43-year period suggests that the same segment 7 was retained throughout the antigenic shifts of HA and NA [ref 10].

Influenza viruses are unique among the respiratory tract viruses in that they undergo significant antigenic variation. Both surface antigens of the influenza A viruses undergo two types of antigenic variation: antigenic drift and antigenic shift. Antigenic drift involves minor antigenic changes in the HA and NA proteins, while antigenic shift involves major antigenic changes in these molecules. Antigenic drift occurs in types A, B, and C virus hemagglutinins but is most pronounced in human influenza A strains. Antigenic drift in the HA occurs by point mutations in the gene, leading to an accumulation of amino acid sequence changes that alter the antigenic sites such that they are no longer recognized by the host's immune system. Antigenic drift also occurs in the NA of influenza viruses and has been correlated with differences in amino acid sequences [ref 11]. The frequency of isolation of NA variants is similar to that for the HA, and sequence analysis shows single amino acid substitutions in the molecule [ref 12]. The humoral immune response in humans is extremely heterogeneous and the limited antibody repertoire against the influenza virus surface antigens in children and young adults probably permits selection of variants with changes in a single epitope [ref 13].

There is no evidence of persistent or latent infection with influenza viruses. The virus is maintained in humans by direct person-to-person spread during acute infection. Immunologic factors clearly influence the size of an epidemic. Immunity is type-specific.

High rates of hospitalization are observed in children less than 5 years of age (undergoing first infection) and those over 65 years of age. The observation that 14-16% of children seeking medical care for febrile respiratory illness are infected by influenza virus indicates that influenza viruses make a major contribution to outpatient morbidity. Immunity to influenza viruses is incomplete and morbidity following reinfection with the same strain or with a drift strain can occur. Although severe disease can attend reinfection, the morbidity following reinfection is usually less than that following first infection.

The economic impact of influenza A virus infection is considerable and morbidity contributes heavily to it. The estimated total cost (direct and indirect costs) of each of three different epidemics varied from 1 to 3 billion dollars depending on the size of the epidemic.

Influenza virus is unique among respiratory viruses in being a cause of excess mortality. Influenza A has caused excess mortality 21 times since 1934. Although morbidity with influenza A virus is concentrated in people less than 20 years of age, the highest rates of excess mortality occur in persons over 65 years of age [ref 14]. Mortality due to influenza is not confined to the bedridden since up to 50% of all deaths occurred in people who were chronically ill but medically stable and functioning independently [ref 15]. The average yearly rate of death due to influenza A virus infection is 1 per 2200 adults 65 years or older [ref 16].

Prior infection with an influenza virus of one serotype bearing highly related internal antigens such as M and NP proteins did not appear to have a major effect on the occurrence or consequences of subsequent infections with influenza viruses belonging to a different subtype. It is reasonable to conclude that immunity to influenza viruses is primary mediated by responses to the surface glycoproteins. Antibody responses to the HA and NA are the major effectors of resistance to reinfection or illness following reinfection. Cellular immune factors also contribute to immunity to influenza A virus infection [ref 16]. Class I-restricted NP-specific CTL amplified from vaccinia-NP recombinant virus-infected animals were capable of a high-level protection following adoptive transfer [ref 17]. In humans, the level of memory class I-restricted CTL present at the time of infection correlated with an enhanced clearance of virus from the respiratory tract [ref 18]. Oligopeptides with amino acid sequences similar to those of the N-terminus of the HA2 polypeptide have antiviral activity against influenza A virus in vitro [ref 19]. The oligopeptides probably act by

interfering with the function of the N-terminus of the HA2 polypeptide.

Inactivated influenza A and B virus vaccines are licensed for parenteral administration in humans. The vaccines currently in use are designated whole-virus (WV) vaccines or split-product (SP) virus vaccine. The WV vaccines contain intact, formalin-treated virus while the SP vaccines contain purified formalin-treated virus disrupted with chemicals that solubilize the lipid-containing viral envelope. Experimental vaccines contain the isolated HA and NA surface proteins. Satisfactory serological responses were achieved in children and adults with doses of vaccine that caused minimal clinical reactions. Local or systemic allergic reactions occur approximately once in every 3900 vaccinations [ref 20]. Intradermal administration offers no advantage over parenteral administration [ref 21]. Parenteral vaccination with influenza A and B viruses consistently induces resistance to illness and, to a lesser extent, infection with influenza A and B virus [ref 22, 23]. Efficacy ranges from 60 to 80%, the higher value being obtained following challenge with homologous virus. Vaccine is effective in the elderly who are at high risk for morbidity and mortality, but the level of efficacy is often less than seen in young adults [ref 15, 24]. It is suggested that the immunity is of short duration.

Inactivated vaccines stimulate HLA-restricted CTL in adults but their ability to stimulate CTL in unprimed individuals has not been evaluated [ref 25]. In contrast to virus infection, inactivated WV and SP vaccines stimulate a poor CTL response in unprimed mice.

Although influenza A viruses do not differ in mutation rate from other RNA viruses, mutation occurs sufficiently often to provide an ample source for the selection of naturally occurring variants. An important part of the resistance to influenza A virus is mediated by the development of an immune response to HA and NA. These glycoproteins are conserved to a much lesser extent compared to the NP and M proteins.

We conceived that newly characterized influenza virus T-cell epitopes of highly conserved proteins may be valuable tools for vaccine development and diagnostic applications. Therefore we focused on the highly conserved influenza M protein.

This invention is specifically concerned with the detection of class I binding T cell epitopes of the highly conserved M protein of the influenza virus, which can be recognized by CTL. CTL are of crucial importance in the resistance against virus infections [ref 26, 27]. CTL specific for viruses (or tumors) recognize short viral or tumor protein-derived peptides of about 8-11 amino acids in length, that are presented in the antigen binding groove of major histocompatibility complex (MHC) class I molecules [ref 28, 29]. Recently in several systems vaccination with peptides recognized by antigen specific CTL was shown to prevent lethal virus infection and to delay tumor growth in mice, whereas adoptive transfer of these CTL could induce tumor destruction [ref 30, 31].

We have succeeded in the identification of influenza virus peptides derived from the highly conserved M protein that bind to the groove of MHC class I molecules, using the antigen processing defective cell line 174.CEM T2 (T2). This cell line expresses the human MHC class I HLA-A2.1 and HLA-B5 alleles of which only the HLA-A2.1 molecules are expressed as partly empty and unstable molecules that can be stabilized on the cell surface with exogenous addition of binding peptides [ref 32]. If incubation with a peptide results in an increased expression of the HLA-A2.1 on the surface of T2, this implies that the peptide binds to the groove of this MHC class I molecule and is therefore a candidate target antigen for recognition by CTL. In the case of HLA-A2.1, it is known that about 40% of the Caucasoid population in Western Europe expresses this allele.

Using an arbitrary computer scoring system developed by ourselves based on a peptide motif rule developed by others, we screened the amino acid sequences of the influenza M protein [ref 33, 34]. In this scoring system we scored 6 points for an anchor residue, 4 points for a strong residue and 2 points for a

weak residue. The score for a given peptide is obtained by multiplication of the scores for each amino acid position. We generated a series of 77 peptides (37 peptides having a length of 9 amino acids, i.e. 9-mers, 24 10-mers, and 16 11-mers) comprising 35 high scoring peptides, 24 low scoring peptides and 18 randomly chosen control peptides. In total 15 peptides of this set were identified to bind to the HLA-A2.1 molecule at a concentration of 100 µg/ml (Table 2). All HLA-A2.1 binding peptides had a high score using the computer program, none of the low-scoring or control peptides did so (Table 3, 4, 5).

One of the HLA-A2.1 binding peptides of the influenza M protein has previously been described (aa 58-66, Table 2). An HLA-A2.1 restricted CTL recognizes this defined influenza M epitope [ref 35].

Summary of the invention

An object of the present invention is therefore to provide synthetic peptides of the influenza M protein for prevention, prophylaxis, therapy and treatment of influenza utilizing these synthetic peptides and pharmaceutical compositions containing the synthetic peptides.

Another object of the invention is to provide a method of prophylactic or therapeutic treatment of influenza or other Human Influenza Virus-related diseases.

A further object of the present invention is to provide pharmaceutical compositions which can be used for prevention, prophylaxis, therapy and treatment of influenza or other Human Influenza Virus-related diseases.

This invention provides a peptide comprising an amino acid sequence derived from a human influenza virus protein, wherein said amino acid sequence has the ability to bind to a human Major Histocompatibility Complex (MHC) Class I molecule.

The present invention also provides specific peptides derived from the amino acid sequence of the human influenza M protein which, because of their capability to bind to HLA molecules, such

as e.g. the HLA-A2.1, HLA-A1, HLA-A3.2, HLA-A11.2 or HLA-A24 protein, are candidate peptides to be included in human vaccines that can induce protective or therapeutic T cell responses against human influenza virus.

The novel peptides of the present invention are useful in pharmaceutical compositions, as screening tools and in the prevention, prophylaxis, therapy and treatment of influenza or other human influenza virus-induced diseases or other conditions which would benefit from inhibition of influenza virus infection.

Preferably, said amino acid sequence has the ability to bind to human MHC Class I allele HLA-A2.1.

More specifically, this invention provides a peptide comprising an amino acid sequence derived from human influenza M protein, wherein said amino acid sequence has the ability to bind to human MHC Class I allele HLA-A2.1 and is selected from the group consisting of:

Peptide	Amino acid sequence	location in influenza M protein
1	S L L T E V E T Y V	(residues 2-11 of M protein)
2	S L L T E V E T Y V L	(residues 2-12 of M protein)
3	L L T E V E T Y V	(residues 3-11 of M protein)
4	L L T E V E T Y V L	(residues 3-12 of M protein)
5	V L M E W L K T R P I	(residues 41-51 of M protein)
6	P I L S P L T K G I	(residues 50-59 of M protein)
7	I L S P L T K G I	(residues 51-59 of M protein)
8	I L S P L T K G I L	(residues 51-60 of M protein)
9	G I L G F V F T L	(residues 58-66 of M protein)
10	G I L G F V F T L T V	(residues 58-68 of M protein)
11	I L G F V F T L T V	(residues 59-68 of M protein)
12	R M G A V T T E V	(residues 134-142 of M protein)
13	G L V C A T C E Q I A	(residues 145-155 of M protein)
14	Q M V T T T N P L	(residues 164-172 of M protein)
15	Q M V T T T N P L I	(residues 164-173 of M protein),

and a fragment, homolog, isoform, derivative, genetic variant or conservative variant of any one of said amino acid sequences

which has the ability to bind to human MHC Class I allele HLA-A2.1.

This invention further provides a pharmaceutical composition containing a prophylactically or therapeutically effective amount of a peptide according to the invention, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant. Preferably, said pharmaceutical composition contains a peptide according to the invention which is able to induce a T cell response effective against human influenza virus, in particular a HLA class I-restricted $CD8^+$ cytotoxic T cell response.

In addition, this invention provides a method of prophylactic or therapeutic treatment of influenza or any other human influenza virus-related disease with a human individual, comprising administering to said human individual a prophylactically or therapeutically effective amount of a peptide according to the invention, more specifically an immunogenic form of a peptide according to the invention which is able to induce a T cell response effective against human influenza virus, in particular a HLA class I-restricted $CD8^+$ cytotoxic T cell response.

The invention provides a peptide as defined herein for prophylactically or therapeutically inducing in a human individual a HLA class I-restricted $CD8^+$ cytotoxic T cell response effective against human influenza virus.

The invention covers the use of a peptide as defined herein for preparing a pharmaceutical composition for prophylactically or therapeutically inducing in a human individual a HLA class I-restricted $CD8^+$ cytotoxic T cell response effective against human influenza virus.

The invention also provides a process of inducing a human influenza virus-specific cytotoxic T lymphocyte response in a T-lymphocyte culture, comprising the steps of loading antigen-presenting cells which carry empty MHC Class I molecules with a peptide as defined herein, culturing T-lymphocytes in the presence of the peptide-loaded antigen-presenting cells under specific cytotoxic T-lymphocyte response-inducing conditions, and, optionally, isolating a human influenza virus-specific

cytotoxic T lymphocyte from the resulting culture and culturing said isolated cytotoxic T-lymphocyte.

The invention provides a human influenza virus-specific cytotoxic T lymphocyte reactive to a peptide as defined herein, and also provides a pharmaceutical composition containing a prophylactically or therapeutically effective amount of a human influenza virus-specific cytotoxic T lymphocyte reactive to a peptide as defined herein, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

The invention also provides a method of prophylactic or therapeutic treatment of a human influenza virus-related disease with a human individual, comprising administering to said human individual a prophylactically or therapeutically effective amount of a human influenza virus-specific cytotoxic T lymphocyte reactive to a peptide as defined herein.

The invention covers the use of a peptide as defined herein in a diagnostic test or assay to detect human influenza virus-specific T cells or antibodies.

Detailed description of the invention

The invention is directed to peptides comprising an amino acid sequence derived from a human influenza virus protein, in particular the human influenza M protein, wherein said amino acid sequence has the ability to bind to a human MHC class I molecule, preferably a human HLA-A2.1 class I molecule. More specifically, such peptides comprise the following amino acid sequences derived from the noted regions of the human influenza virus (See Table 2; the amino acids are identified by the one-letter code of amino acids).

Table 2: Influenza matrix peptides identified as peptides binding to HLA-A2.1

Peptide SEQ ID NO	Amino acid sequence	location in influenza M protein
1	S L L T E V E T Y V	(residues 2-11 of M protein)
2	S L L T E V E T Y V L	(residues 2-12 of M protein)
3	L L T E V E T Y V	(residues 3-11 of M protein)
4	L L T E V E T Y V L	(residues 3-12 of M protein)
5	V L M E W L K T R P I	(residues 41-51 of M protein)
6	P I L S P L T K G I	(residues 50-59 of M protein)
7	I L S P L T K G I	(residues 51-59 of M protein)
8	I L S P L T K G I L	(residues 51-60 of M protein)
9	G I L G F V F T L	(residues 58-66 of M protein)
10	G I L G F V F T L T V	(residues 58-68 of M protein)
11	I L G F V F T L T V	(residues 59-68 of M protein)
12	R M G A V T T E V	(residues 134-142 of M protein)
13	G L V C A T C E Q I A	(residues 145-155 of M protein)
14	Q M V T T T N P L	(residues 164-172 of M protein)
15	Q M V T T T N P L I	(residues 164-173 of M protein)

The data suggest that the peptides mentioned above are single polypeptides of identified sequences. However, homologs, isoforms or genetic variants of these peptides may exist within or outside the cellular environment. This invention encompasses all such homologs, isoforms or genetic variants of the above peptides provided that they bind to the HLA molecule in question.

Polypeptides that are homologs of the peptides specifically include those having amino acid sequences which are at least about 40% conserved in relation to the amino acid sequence set forth in Table 2, preferentially at least about 60% conserved, and more preferentially at least about 75% conserved.

It will be understood by one of ordinary skill in the art that other variants of the peptides shown above are included within the scope of the present invention. This particularly

includes any variants that differ from the above mentioned and synthesized peptides only by conservative amino acid substitution. In particular, replacements of C (cysteine) by A (alanine), S (serine), α-aminobutyric acid and others are included as it is known that cysteine-containing peptides are susceptible to (air) oxidation during synthesis and handling. Many such conservative amino acid substitutions are set forth as sets by Taylor (1986).

Peptides according to the invention, or fragments thereof, include any variation in the amino acid sequence, whether by conservative amino acid substitution, deletion, or other process, provided that the polypeptides bind to the HLA molecule in question. The fragments of the peptides may be small peptides with sequences of as little as five or more amino acids, said sequences being those disclosed in Table 2 when said polypeptides bind to the HLA-A2.1 molecule.

Polypeptides larger than peptides Nos. 1-15 are especially included within the scope of the present invention when said polypeptides induce a human influenza M protein specific CTL response in appropriate individuals (e.g. HLA-A2.1 positive individuals) and include a (partial) amino acid sequence as set forth in Table 2, or conservative substitutions thereof. Such polypeptides may have a length up to about 30 amino acids, preferably up to about 27 amino acids. Most preferably, however, the peptides have a length of from 9 to 12, more preferably 9 to 11 amino acids.

This invention includes the use of polypeptides Nos. 1-15 generated by every means, whether genetic engineering, peptide synthesis with solid phase techniques or others. The peptides may have various chemical modifications made at the terminal ends and still be within the scope of the present invention. Also other chemical modifications are possible, particularly cyclic and dimeric configurations. The term "derivatives" intends to cover all such modified peptides.

The polypeptides of the present invention find utility for the treatment or prevention of diseases involving the human influenza virus.

For all applications the peptides are administered in an immunogenic form. Since the peptides are relatively short, this may necessitate conjugation with a immunogenicity conferring carrier material such as lipids or others or the use of adjuvants.

The magnitude of a prophylactic or therapeutic dose of polypeptides of this invention will, of course, vary with the group of patients (age, sex, weight, etcetera), the nature of the severity or the condition to be treated, the particular polypeptide of this invention and its route of administration. Any suitable route of administration may be employed to achieve an effective dosage of a polypeptide identified by this invention, as well as any dosage form well known in the art of pharmacy. In addition the polypeptides may also be administered by controlled release means and/or other delivery devices, such as, in particular, interferons and T cell activating agents like interleukin-2 etc.

The peptides of this invention may also be useful for other purposes, such as diagnostic use. For example, they may be used to check whether a vaccination with a peptide according to the present invention has been successful. This may be done in vitro by testing whether the peptide is able to activate T cells of the vaccinated person. They may be used also in diagnostic tests or assays for the detection of human influenza virus-specific antibodies.

The following example illustrates the present invention.

EXAMPLE

In order to identify human influenza M protein peptides that can bind to the groove of HLA-A2.1 molecules, the amino acid sequence of human influenza M protein was examined with an arbitrary computer scoring system developed by ourselves based on a peptide motif rule developed by others [ref 33, 34]. In our scoring system we gave 6 points for an anchor residue, 4 points for a strong residue and 2 points for a weak residue. The score

for a given peptide is obtained by multiplication of the scores for each amino acid position.

We generated a set of 77 peptides (37 of them were 9-mers, 24 were 10-mers, and 16 were 11-mers) comprising 35 high scoring peptides, 24 low scoring peptides and 18 randomly chosen control peptides. Peptides having a length of 9, 10 or 11 amino acids were chosen because they fit the presently known rules for the length of peptides that bind to the groove of HLA-A2.1 molecules.

Peptides were synthesized by solid phase strategies on an automated multiple peptide synthesizer (Abimed AMS 422) using Fmoc-chemistry. The purity of the peptides was determined by reversed phase HPLC. Peptides were dissolved in DMSO (final DMSO concentration 0.25%) and diluted in 0.9% NaCl to a peptide concentration of 2 mg/ml and stored at -20°C.

To determine which peptides were able to bind to the groove of MHC class I molecules, we used the antigen processing defective cell line 174.CEM T2 (T2). This cell line expresses the human MHC class I HLA-A2.1 and HLA-B5 alleles of which only the HLA-A2.1 molecules are expressed as partly empty and unstable molecules that can be stabilized on the cell surface with exogenous addition of peptides binding to the peptide presenting groove of these molecules [ref 32]. If incubation with a peptide results in an increased expression of the HLA-A2.1 on the surface of T2, this implies that the peptide binds to the groove of this MHC class I molecule and is therefore a candidate target antigen for recognition by CTL.

The T2 cell line, a gift from Dr. P. Cresswell (Dept. of Immunology, Yale University, New Haven, CT), was cultured in Iscove's modified Dulbecco's medium (IMDM) (Biochrom KG, Seromed Berlin, Germany) with 2mM glutamine, 100 IU/ml penicillin, 100 µg/ml kanamycin and 10% fetal calf serum (FCS, Hyclone Laboratories Inc. Logan, Utah, USA). The T2 cells were washed twice in serum free culture medium. 80,000 Cells in 40 µl serum free medium were put into a V bottomed 96 well plate together with 10 µl of the peptide dilution at different concentrations

or 10 µl 0.9% NaCl, and incubated overnight at 37°C, 5% CO_2 in humidified air. The cells were washed once with cold (4°C) "PBA" (0.9% NaCl, 0.5% BSA, 0.02% NaN_3) and stained by indirect immunofluorescence. The first antibody BB7.2 (HLA-A2.1 specific monoclonal antibody) was added in a saturating amount and incubated for 30 minutes at 4°C. After two wash steps with cold PBA, FITC-labelled $F(ab')_2$ fragments of goat anti-mouse IgG (Tago Inc Burlinger, CA, USA: 4350) in a dilution of 1:40 was added as the second antibody and incubated for another 30 min at 4°C. The cells were washed once and fluorescence was measured at 488 nanometer on a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

The fluorescence Ratio (F.R.) was calculated according to the formula:

F.R. = mean fluorescence experimental sample / mean fluorescence background.

Only the 15 peptides described in Table 2 were able, at a concentration of 100 µg/ml, to significantly upregulate the expression of HLA-A2.1 molecules measured as mean HLA-A2.1 fluorescence of T2 cells indicating their binding to the HLA-A2.1 molecule. None of the other human influenza M protein peptides were able to do this. All of the HLA-A2.1 binding peptides had a high score in our scoring system.

These experiments indicate that only a limited proportion of peptides (Nos. 1-15) have the ability to bind to the HLA-A2.1 molecule and are therefore the candidates of the human influenza M protein to be recognized by human CTL because CTL recognize peptides only when bound to the groove of HLA molecules. Furthermore, they are the candidates to be used for either vaccination of human beings in the prevention or therapy of diseases with expression of influenza M protein, or for the in vitro response induction of influenza virus specific CTL.

Our elucidation of peptides derived from the human influenza M protein which bind to human MHC Class I allele HLA-A2.1 allows the formulation of powerful vaccines against human influenza virus-related conditions, such as influenza. Until our

invention, only one CTL epitope of the influenza M protein had been elucidated (the immunodominant epitope aa 58-66).

It is known from studies in mice performed in our group that a peptide representing a subdominant epitope of a tumor cell line can protect mice vaccinated with said peptide against outgrowth of that cell line, although vaccination with tumor cells does not elicit a response against this peptide. We assume that in addition to the immunodominant epitope of the influenza M protein (aa 58-66) the other binding peptides of this protein can have an important immunologic function if used in a vaccine.

The 77 peptides synthesized and tested are shown in Table 2 (15 high-scoring and binding influenza matrix peptides), Table 3 (20 high-scoring but non-binding influenza matrix peptides), Table 4 (24 low-scoring, non-binding influenza matrix peptides) and Table 5 (18 non-binding control influenza matrix peptides).

The result of the binding analyses of the 15 binding peptides is given in Table 6. Binding of peptides is shown as mean fluorescence level of HLA-A2.1 expression in the presence of said peptides divided by the mean fluorescence level of HLA-A2.1 expression without the presence of said peptides. Binding of a peptide is regarded as positive when a value of 1.5 is reached.

Table 3: The non-binding influenza matrix peptides with a high score. The number of the first amino acid is shown. The peptides are ranked according to their computer score.

First aa No.	score	peptides
14	1152	I I P S G P L K A
14	1152	I I P S G P L K A E I
54	576	P L T K G I L G F V
98	576	K L Y R K L K R E I
106	576	E I T F H G A K E I
50	288	P I L S P L T K G I L
134	288	R M G A V T T E V A
218	288	R I G T H P S S S A
11	144	V L D I I P S G P L
19	144	P L K A E I A Q R L
23	144	E I A Q R L E D V
23	144	E I A Q R L E D V F A
102	144	K L K R E I T F H G A
130	144	L I Y N R M G A V
145	144	G L V C A T C E Q I
172	144	L I R H E N R M V
172	144	L I R H E N R M V L
172	144	L I R H E N R M V L A
191	144	Q M A G S S E Q A
228	144	G L K N D L L E N L

Table 4: The Influenza matrix peptides with a low score. The number of the first amino acid is shown. The peptides are ranked according to their computer score.

First aa No.	score	peptides
13	72	D I I P S G P L K A
42	72	L M E W L K T R P I
42	72	L M E W L K T R P I L
45	72	W L K T R P I L S P L
114	72	E I S L S Y S A G A
114	72	E I S L S Y S A G A L
116	72	S L S Y S A G A L
116	72	S L S Y S A G A L A
123	72	A L A S C M G L I
127	72	C M G L I Y N R M
127	72	C M G L I Y N R M G A
129	72	G L I Y N R M G A
129	72	G L I Y N R M G A V
171	72	P L I R H E N R M
171	72	P L I R H E N R M V
171	72	P L I R H E N R M V L
236	72	N L Q A Y Q K R M
236	72	N L Q A Y Q K R M G V
38	36	D L E V L M E W L
65	36	T L T V P S E R G L
73	36	G L Q R R R F V Q N A
178	36	R M V L A S T A K A
211	36	Q M V Q A M I R I
180	24	V L A S T A K A M

Table 5: The Influenza matrix peptides used as a control group. The number of the first amino acid is shown. The peptides are ranked according to their seq. no.

First aa No.	peptides
20	L K A E I A Q R L
31	V F A G K N T D L
41	V L M E W L K T R
47	K T R P I L S P L
55	L T K G I L G F V
60	L G F V F T L T V
75	Q R R R F V Q N A
88	G D P N N M D K A
91	N N M D K A V K L
95	K A V K L Y R K L
98	K L Y R K L K R E
99	L Y R K L K R E I
107	I T F H G A K E I
173	I R H E N R M V L
179	M V L A S T A K A
194	G S S E Q A A E A
201	E A M E V A S Q A
232	D L L E N L Q A Y

Table 6

No.	First aa No.	score	peptides	F.R.
1	2	288	S L L T E V E T Y V	2.38
2	2	288	S L L T E V E T Y V L	3.51
3	3	576	L L T E V E T Y V	2.69
4	3	576	L L T E V E T Y V L	3.11
5	41	1152	V L M E W L K T R P I	2.79
6	50	288	P I L S P L T K G I	1.66
7	51	576	I L S P L T K G I	2.73
8	51	576	I L S P L T K G I L	1.56
9	58	576	G I L G F V F T L	2.92
10	58	576	G I L G F V F T L T V	2.14
11	59	144	I L G F V F T L T V	1.87
12	134	288	R M G A V T T E V	2.12
13	145	144	G L V C A T C E Q I A	1.83
14	164	144	Q M V T T T N P L	3.31
15	164	144	Q M V T T T N P L I	1.98

SEQUENCE LISTING

SEQ ID NO:1
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 10 amino acids
SerLeuLeuThrGluValGluThrTyrVal
1 5 10

SEQ ID NO:2
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 11 amino acids
SerLeuLeuThrGluValGluThrTyrValLeu
1 5 10

SEQ ID NO:3
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 9 amino acids
LeuLeuThrGluValGluThrTyrVal
1 5

SEQ ID NO:4
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 10 amino acids
LeuLeuThrGluValGluThrTyrValLeu
1 5 10

SEQ ID NO:5
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 11 amino acids
ValLeuMetGluTrpLeuLysThrArgProIle
1 5 10

SEQ ID NO:6
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 10 amino acids
ProIleLeuSerProLeuThrLysGlyIle
1 5 10

SEQ ID NO:7
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 9 amino acids
IleLeuSerProLeuThrLysGlyIle
1 5

SEQ ID NO:8
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 10 amino acids
IleLeuSerProLeuThrLysGlyIleLeu
1 5 10

SEQ ID NO:9
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 9 amino acids
GlyIleLeuGlyPheValPheThrLeu
1 5

SEQ ID NO:10
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 11 amino acids
GlyIleLeuGlyPheValPheThrLeuThrVal
1 5 10

SEQ ID NO:11
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 10 amino acids
IleLeuGlyPheValPheThrLeuThrVal
1 5 10

SEQ ID NO:12
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 9 amino acids
ArgMetGlyAlaValThrThrGluVal
1 5

SEQ ID NO:13
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 11 amino acids
GlyLeuValCysAlaThrCysGluGlnIleAla
1 5 10

SEQ ID NO:14
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 9 amino acids
GlnMetValThrThrThrAsnProLeu
1 5

SEQ ID NO:15
SEQUENCE TYPE: amino acid
SEQUENCE LENGTH: 10 amino acids
GlnMetValThrThrThrAsnProLeuIle
1 5 10

References

1. Smith W., Andrewes C.H., Laidlaw P.P. A virus obtained from influenza patients. Lancet 1933; i: 66-68.
2. Lamb R.A. The genes and proteins of the influenza viruses. In: Krug RM, ed. The influenza viruses. New York: Plenum, 1989.
3. Wiley D.C., Skehel J.J. The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. Annu. Rev. Biochem. 1987; 56: 365-394.
4. Bastin J., Rothbard J., Davey J., Jones I., Townsend A. Use of synthetic peptides of influenza nucleoprotein to define epitopes recognized by class I-restricted cytotoxic T lymphocytes. J. Exp. Med. 1987; 165: 1508-1523.
5. Yewdell J.W., Hackett C.J. The specificity and function of T lymphocytes induced by influenza A viruses. In: Krug R, ed. The influenza viruses. New York: Plenum Press, 1989.
6. Joassin L., Vincenzotto C., Cloes J.-M., Bouchet M., Reginster M. Monoclonocal antibodies detect M-protein epitopes on the surface of influenza virions. Arch. Virol. 1987; 95: 183-195.
7. Biddison W.E., Doherty P.C., Webster R.G. Antibody to influenza virus matrix protein detects a common antigen on the surface of cells infected with type A influenza viruses. J. Exp. Med. 1977; 146: 690-697.
8. Ye Z., Pal R., Fox J.W., Wagner R.R. Functional and antigenic domains of the matrix (M) protein of influenza virus. J. Virol. 1987; 61: 239-246.
9. Askonas B.A., McMichael A.J., Webster R.G. The immune response to influenza viruses and the problem of protection against infection. In: Beare A.S., ed. Basic and applied influenza research. Boca Raton, Fl: CRC Pres, 1982; 159-188.
10. Hall R.M., Air G.M. Variation in nucleotide sequences coding for the N-terminal regions of the matrix and non-structural proteins of influenza A viruses. J. Virol. 1981; 38: 1-7.

11. Colman P.M., Ward C.W. Structure and diversity of influenza virus neuraminidase. Curr. Top. Microbiol. Immunol. 1985; 11: 177-255.
12. Laver W.G., Air G.M., Webster R.G., Markoff L.J. Amino acid sequence changes in antigenic variants of type A influenza virus N2 neuraminidase. Virology 1982; 122: 450-460.
13. Wang M.-L., Skehel J.J., Wiley D.C. Comparative analyses of the specificities of anti-influenza hemagglutinin antibodies in human sera. J. Virol. 1986; 57: 124-128.
14. Barker W.H. Excess pneumonia and influenza associated hospitalization during influenza epidemics in the United States. Am. J. Public Health 1986; 76: 761-765.
15. Barker W.H., Mullooly J.P. Influenza vaccination of elderly persons. Reduction in pneumonia and influenza hospitalizations and deaths. JAMA 1980; 244: 2547-2549.
16. Alling D.W., Blackwelder W.C., Stuart-Harris C.H. A study of excess mortality during influenza epidemics in the United States, 1968-1976. Am. J. Epidemiol. 1981; 113: 30-43.
17. Wraith D.C., Vessey A.E., Askonas B.A. Purified influenza virus nucleoprotein protects mice from lethal infection. J. Gen. Virol. 1987; 68: 433-440.
18. McMichael A.J., Gotch F.M., Noble G.R., Beare P.A.S. Cytotoxic T-cell immunity of influenza. N. Engl. J. Med. 1983; 309: 13-17.
19. Richardson C.D., Scheid A., Choppin P.W. Specific inhibition of paramyxovirus and myxovirus replication by oligopeptides with amino acid sequences similar to those at the N-termini of the F1 or HA2 viral polypeptides. Virology 1980; 105: 205-222.
20. Davies R., Pepys J. Egg allergy, influenza vaccine, and immunoglobulin E antibody. J. Allergy Clin. Immunol. 1976; 57: 373-383.
21. Nicholson K.G., Tyrrell D.A.J., Harrison P. et al. Clinical studies of monovalent inactivated whole virus and subunit A/USSR/77 (HINI) vaccine: serological responses and clinical reactions. J. Biol. Stand. 1979; 7: 123-136.

22. Feery B.J., Evered M.G., Morrison E.I. Different protection rates in various groups of volunteers given subunit influenza virus vaccine in 1976. J. Infect. Dis. 1979; 139: 237-241.
23. Meiklejohn G., Eickboff T.C., Graves P., Josephine I. Antigenic drift and efficacy of influenza virus vaccines, 1976-1977. J. Infect. Dis. 1978; 138: 618-624.
24. Strassburg M.A., Greenland S., Sorviollo F.J., Lieb L.E., Habel L.A. Influenza in the elderly: Report of an outbreak and a review of vaccine effectiveness report. Vaccine 1987; 38-44.
25. Ennis F.A., Rook A.H., Hua Q.Y. et al. HLA-restricted virus specific cytotoxic T-lymphocyte responses to live and inactivated influenza vaccines. Lancet 1981; ii: 887-891.
26. Melief C.J.M. Tumor eradication by adoptive transfer of cytotoxic T lymphocytes. Adv. in Cancer Res. 1992; 58: 143-175.
27. Kast W.M., Melief C.J.M. In vivo efficacy of virus-derived peptides and virus-specific cytotoxic T lymphocytes. Immunol. Letters 1991; 30: 229-232.
28. Townsend A., Öhlen C., Bastin, Ljunggren H.G., Foster L., Kärre K. Association of class I major histocompatibility heavy and light chains induced by viral peptides. Nature 1989; 340: 443-450.
29. Bjorkman P.J., Saper M.A., Samraoui B., Bennet W.S., Strominger J.L., Wiley D.C. Structure of the human class I histocompatibility antigen, HLA-A2 Nature 1987; 329: 506-521.
30. Kast W.M., Offringa R., Peters P.J. et al. Eradication of adenovirus E1-induced tumours by E1A-specific cytotoxic T lymphocytes. Cell 1989; 59: 603-614.
31. Schulz M., Zinkernagel R. and Hengartner. Peptide-induced antiviral protection by cytotoxic T cells. PNAS 1991; 88: 991-993.
32. Salter R.D., Cresswell P. Impaired assembly and transport of HLA-A and -B antigens in a mutant TxB cell hybrid. EMBO 1986; 5: 943-949.
33. Falk K., Rötzschke O., Stevanovic S., Jung G., Rammensee H.G. Allele-specific motifs revealed by sequencing of self peptides eluted from MHC molecules. Nature 1991; 351: 290-296.

34. Hunt D.F., Henderson R.A., Shabanowitz J. et al. Characterization of peptides bound to the class I MHC molecules HLA-A2.1 by mass spectometry. Science 1992; 255: 1261-1263.
35. Morrison J., Elvin J., Latron et al. Identification of the nonamer peptide form influenza A matrix protein and the role of pockets of HLA-A2 in its recognition by cytotoxic T lymphocytes. Eur. J. Immunol. 1992; 22: 903-907.

CLAIMS

1. A peptide comprising an amino acid sequence derived from a human influenza virus protein, wherein said amino acid sequence has the ability to bind to a human MHC Class I molecule.

2. A peptide according to claim 1, wherein said amino acid sequence is derived from human influenza M protein.

3. A peptide according to claim 1 or claim 2, wherein said amino acid sequence has the ability to bind to human MHC Class I allele HLA-A2.1.

4. A peptide according to claim 1, comprising an amino acid sequence derived from human influenza M protein, wherein said amino acid sequence has the ability to bind to human MHC Class I allele HLA-A2.1 and is selected from the group consisting of:

Peptide	Amino acid sequence	location in influenza M protein
1	S L L T E V E T Y V	(residues 2-11 of M protein)
2	S L L T E V E T Y V L	(residues 2-12 of M protein)
3	L L T E V E T Y V	(residues 3-11 of M protein)
4	L L T E V E T Y V L	(residues 3-12 of M protein)
5	V L M E W L K T R P I	(residues 41-51 of M protein)
6	P I L S P L T K G I	(residues 50-59 of M protein)
7	I L S P L T K G I	(residues 51-59 of M protein)
8	I L S P L T K G I L	(residues 51-60 of M protein)
9	G I L G F V F T L	(residues 58-66 of M protein)
10	G I L G F V F T L T V	(residues 58-68 of M protein)
11	I L G F V F T L T V	(residues 59-68 of M protein)
12	R M G A V T T E V	(residues 134-142 of M protein)
13	G L V C A T C E Q I A	(residues 145-155 of M protein)
14	Q M V T T T N P L	(residues 164-172 of M protein)
15	Q M V T T T N P L I	(residues 164-173 of M protein),

and a fragment, homolog, isoform, derivative, genetic variant or conservative variant of any one of said amino acid sequences which has the ability to bind to human MHC Class I allele HLA-A2.1.

5. A peptide according to any one of the claims 1-4, having a length of from 9 to 12, preferably 9 to 11 amino acids.

6. A pharmaceutical composition containing a prophylactically or therapeutically effective amount of a peptide according to any one of the claims 1-5, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

7. A pharmaceutical composition containing a prophylactically or therapeutically effective amount of a peptide according to any one of the claims 1-5 which is able to induce a T cell response effective against human influenza virus, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

8. A pharmaceutical composition containing a prophylactically or therapeutically effective amount of a peptide according to any one of the claims 1-5 which is able to induce a HLA class I-restricted $CD8^+$ cytotoxic T cell response effective against human influenza virus, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

9. A method of prophylactic or therapeutic treatment of a human influenza virus-related disease with a human individual, comprising administering to said human individual a prophylactically or therapeutically effective amount of a peptide according to any one of the claims 1-5.

10. A method of prophylactic or therapeutic treatment of a human influenza virus-related disease with a human individual, comprising administering to said human individual a prophylactically or therapeutically effective amount of an immunogenic form of a peptide according to any one of the claims 1-5 which is able to induce a T cell response effective against human influenza virus.

11. A method of prophylactic or therapeutic treatment of a human influenza virus-related disease with a human individual, comprising administering to said human individual a prophylactically or therapeutically effective amount of an immunogenic form of a peptide according to any one of the claims 1-5 which is able to induce a HLA class I-restricted $CD8^+$ cytotoxic T cell response effective against human influenza virus.

12. A peptide according to any one of claims 1-5 for prophylactically or therapeutically inducing in a human individual a HLA class I-restricted $CD8^+$ cytotoxic T cell response effective against human influenza virus.

13. Use of a peptide according to any one of claims 1-5 for preparing a pharmaceutical composition for prophylactically or therapeutically inducing in a human individual a HLA class I-restricted $CD8^+$ cytotoxic T cell response effective against human influenza virus.

14. A process of inducing a human influenza virus-specific cytotoxic T lymphocyte response in a T-lymphocyte culture, comprising the steps of loading antigen-presenting cells which carry empty MHC Class I molecules with a peptide according to any one of the claims 1-5, culturing T-lymphocytes in the presence of the peptide-loaded antigen-presenting cells under specific cytotoxic T-lymphocyte response-inducing conditions, and, optionally, isolating a human influenza virus-specific cytotoxic T lymphocyte from the resulting culture and culturing said isolated cytotoxic T-lymphocyte.

15. A human influenza virus-specific cytotoxic T lymphocyte reactive to a peptide according to any one of the claims 1-5.

16. A pharmaceutical composition containing a prophylactically or therapeutically effective amount of a human influenza virus-specific cytotoxic T lymphocyte reactive to a peptide according to any one of the claims 1-5, and a pharmaceutically acceptable carrier, diluent, excipient or adjuvant.

17. A method of prophylactic or therapeutic treatment of a human influenza virus-related disease with a human individual, comprising administering to said human individual a prophylactically or therapeutically effective amount of a human influenza virus-specific cytotoxic T lymphocyte reactive to a peptide according to any one of the claims 1-5.

18. Use of a peptide according to any one of claims 1-5 in a diagnostic test or assay to detect human influenza virus-specific T cells or antibodies.

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(57) Abstract

A peptide comprising an amino acid sequence derived from a human influenza virus protein, such as the human influenza M protein, wherein said amino acid sequence has the ability to bind to a human Major Histocompatibility Complex Class I molecule. Its use in prophylactic or therapeutic treatment of a human influenza virus-related disease, or in a diagnostic test or assay.

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INTERNATIONAL SEARCH REPORT

Inten nal Application No
PCT/NL 94/00114

A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	EUROPEAN JOURNAL OF IMMUNOLOGY, vol.23, no.6, June 1993 pages 1215 - 1219 NIJMAN H. W. ET AL. 'Identification of peptide sequences that potentially trigger HLA-A2.1-restricted cytotoxic T lymphocytes' see the whole document ---	1-17
P,X	HUMAN IMMUNOLOGY, vol.37, no.4, August 1993 pages 252 - 258 SAUMA, S.Y. ET AL. 'Recognition by HLA-A2-restricted cytotoxic T lymphocytes of endogenously generated and exogenously provided synthetic peptide analogues of the Influenza A virus Matrix Protein' see the whole document ---	1-5

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[X] Further documents are listed in the continuation of box C.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	NATURE., vol.351, 23 May 1991, LONDON GB pages 290 - 296 FALK, K. ET AL. 'Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules' cited in the application see page 292, column 2, paragraph 5 - page 296, column 2, line 10; table 4	1-13,17
X	EUROPEAN JOURNAL OF IMMUNOLOGY, vol.22, no.4, 1992, WEINHEIM, BRD pages 903 - 907 MORRISON, J. ET AL. 'Identification of the nonamer peptide from influenza A matrix protein and the role of pockets of HLA-A2 in its recognition by cytotoxic T lymphocytes' cited in the application	1-5,14, 15
A	WO,A,88 08852 (SRI INTERNATIONAL) 17 November 1988 see claims	1,5

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL94/00114

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark : Although claims 9 - 11 and 17 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter nal Application No
PCT/NL 94/00114

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-8808852	17-11-88	US-A-	4981782	01-01-91
		EP-A,B	0313651	03-05-89
		GB-A,B	2213823	23-08-89
		JP-T-	1503464	22-11-89